

## CLAIMS

1. A surface for attachment of molecules comprising:
  - (a) a substrate; and
  - (b) a coating thereon of a homogeneous mixture of
    - a labeled compound
    - and
    - a first chemically functional compound,other molecules being chemically bondable to said functional compound when in said coating.
2. A surface of claim 1, wherein said labeled compound is labeled by a fluorescent moiety.
3. A surface according to claim 2, wherein the substrate is a low self-fluorescent glass.
4. A surface according to claim 3, wherein the glass is borosilicate or soda lime silicate glass.

5. A surface according to claim 2, wherein the fluorescent labeled compound is derived from the reaction of a fluorescent dye and a second chemically functional compound.
6. A surface according to claim 1, wherein the chemically functional compound is an organosilane.
7. A surface according to claim 1, wherein the chemically functional compound is an amino functional monoalkoxy, dialkoxy, trialkoxy or chlorosilane.
8. A surface according to claim 7, wherein the chemically functional compound is an epoxide functionalized alkoxy or chlorosilane.
9. A surface according to claim 2, wherein the chemically functional compound is a multiaminoorganosilane.
10. A surface according to claim 1, wherein the chemically functional compound is an alkylalkoxysilane.
11. A surface according to claim 1, wherein the chemically functional compound is an alkanethiol.

12. A surface according to claim 6, wherein the organosilane is N- (2-aminoethyl)-3-aminopropyltrimethoxysilane (EDA), trimethoxysilylpropyl-diethylenetriamine (DETA), (aminoethyl aminomethyl) phenethyltrimethoxysilane (PEDA), dimethoxysilylpropyldiethylenetriamine, N- (2-aminoethyl)-3-aminopropylmethylmethoxysilane, N- (6-aminoethyl) aminopropyltrimethoxysilane (AHA), 4-aminobutyltriethoxysilane, N- (2-aminoethyl)-3-aminoisobutylmethylmethoxysilane, or a mixture thereof.

13. A surface according to claim 8, wherein the epoxysilane is 2-(3,4-epoxycyclohexyl) ethyltrimethoxysilane, (3-glycidoxypropyl) trimethoxysilane, (3-glycidoxy propyl) dimethoxysilane, (3-glycidoxy propyl) methyldiethoxysilane, (3-glycidoxy propyl) methylmethoxysilane, 2-(3,4-epoxycyclohexyl) ethyltriethoxysilane, 5,6-epoxyhexyltriethoxysilane, or a mixture thereof.

14. A surface according to claim 5, wherein the fluorescent compound is (4-methylcoumarinyl)-N-[3- triethoxysilylpropyl] carbonate, (triethoxysilylpropyl) dansylamide, 4-methyl-3-trimethylsiloxycoumarin; 3-(2,4-dinitrophenylamino) propyltriethoxysilane, N-triethoxysilylpropylquinineurethane, 3-(2,4-dinitrophenylamino) propyltriethoxysilane, or a mixture thereof.

15. An array of immobilized biomolecules comprising a plurality of biomolecules attached to a surface according to claim 2.

16. An array of immobilized nucleic acid molecules comprising a plurality of nucleic acid molecules attached to a surface according to claim 2.

17. A method of performing a hybridization assay comprising incubating target nucleic acid molecules with a surface according to claim 15, wherein the biomolecules are nucleic acid oligomers.

18. An array according to claim 15, wherein the biomolecules are modified or unmodified nucleic acids, antibodies, antigens, proteins, sugars, carbohydrates or oligonucleotides.

19. A substrate according to claim 2, wherein said fluorescent labeled compound is 100%-1% by weight of the homogeneous coating mixture.

20. A substrate according to claim 19, wherein said fluorescent labeled compound is less than 25% by weight of the homogeneous coating mixture.

21. A substrate according to claim 1, wherein said chemically functional compound is at least 1% by weight of the homogeneous coating mixture.

22. A substrate according to claim 1, wherein the homogeneous coating is applied to the substrate by chemical vapor deposition, sputtering, dip coating, spin coating, ion beam deposition, flame hydrolysis deposition, laser pyrolysis deposition, liquid phase deposition, electron beam deposition, plasma arc deposition or evaporation deposition.

23. An array according to claim 15, wherein said chemically functional compound is an alkylalkoxysilane, an amino silane, a hydrogel, an epoxysilane or an alkane thiol.

24. A surface according to claim 2, wherein said fluorescent-labeled compound does not affect the biofunctional properties of the coated bioarray substrate.

25. A surface according to claim 3, wherein said glass comprises, in % by weight on an oxide basis:

SiO <sub>2</sub>	58-85
B <sub>2</sub> O <sub>3</sub>	7 –15
Al <sub>2</sub> O <sub>3</sub>	0 – 8
Na <sub>2</sub> O	0- 15
K <sub>2</sub> O	0 – 8
ZnO	0 – 8

CaO            0 – 8

MgO            0 – 8

As<sub>2</sub>O<sub>3</sub>          0-2

Sb<sub>2</sub>O<sub>3</sub>          0-2.

26. A surface according to claim 3, wherein said glass comprises, in % by weight on an oxide basis:

SiO<sub>2</sub>            40 - 60

B<sub>2</sub>O<sub>3</sub>            10-20

Al<sub>2</sub>O<sub>3</sub>           8-20

BaO             20-30

Na<sub>2</sub>O            0-5

K<sub>2</sub>O             0-5

ZnO             0-7

CaO             0-8

MgO             0-5

As<sub>2</sub>O<sub>3</sub>          0-2

Sb<sub>2</sub>O<sub>3</sub>          0-2.

27. A surface according to claim 3, wherein said glass comprises, in % by weight on an oxide basis:

SiO<sub>2</sub>            60-70

B<sub>2</sub>O<sub>3</sub>            5-10

$\text{Al}_2\text{O}_3$	0.1-8
$\text{Na}_2\text{O}$	0-8
$\text{K}_2\text{O}$	0-8
$\text{ZnO}$	3-10
$\text{TiO}_2$	1-10
$\text{CaO}$	0-5
$\text{MgO}$	0-5
$\text{As}_2\text{O}_3$	0-2
$\text{Sb}_2\text{O}_3$	0-2.

28. A surface according to claim 3, wherein said glass comprises, in % by weight on an oxide basis:

$\text{SiO}_2$	65-75
$\text{Na}_2\text{O}$	5-15
$\text{K}_2\text{O}$	5-15
$\text{ZnO}$	2-6
$\text{TiO}_2$	0.1-5
$\text{BaO}$	0.1-5
$\text{CaO}$	0-10
$\text{MgO}$	0-6
$\text{PbO}$	0-3
$\text{Al}_2\text{O}_3$	0-3
$\text{B}_2\text{O}_3$	0-5

As<sub>2</sub>O<sub>3</sub>                      0-2

Sb<sub>2</sub>O<sub>3</sub>                      0-2.

29.    An array of immobilized carbohydrate molecules comprising a plurality of carbohydrate molecules attached to a surface according to claim 1.

30.    An array of immobilized protein molecules comprising a plurality of protein molecules attached to a surface according to claim 1.

31.    An array of immobilized cells comprising a plurality of cells attached to a surface according to claim 1.

32.    An array of immobilized small molecules comprising a plurality of small molecules attached to a surface according to claim 1.

33.    A surface according to claim 1, where said substrate is gold-coated.

34.    A surface according to claim 28, wherein the substrate is gold coated glass, gold coated ceramic, gold coated glass-ceramic or a gold coated polymeric substrate.

35.    A non-destructive method of determining the uniformity of a coating on a substrate comprising:



detecting the presence and locations of a labeled compound on a substrate which has been coated with a homogenous coating mixture comprising

a labeled compound

and

a chemically functional compound

other molecules being chemically bondable to said functional compound when in said coating.

36. A method of claim 35 wherein the label is a fluorescent moiety and said detecting comprises subjecting the coated substrate to radiant energy to cause the fluorescent labeled compound to emit in the 300 -520 nm wavelength range.
37. A method of claim 35 wherein the label is a fluorescent moiety and said detecting comprises subjecting the coated substrate to IR energy to cause the fluorescent labeled compound to emit in the >700nm wavelength range.
38. A method as in claim 36, wherein said fluorescent labeled compound is 100%-1% by weight of the homogenous coating mixture.

39. A method as in claim 36, wherein said chemically functional compound is an alkanethiol.

40. A method as in claim 35, wherein said chemically functional compound is an alkylalkoxysilane.

41. A method as in claim 36, wherein said substrate comprises chemical moieties for conducting a bioassay and said fluorescent labeled compound does not affect the biofunctional properties of the coated substrate.

42. A method as in claim 36, wherein said substrate is glass.

43. The method of claim 36, wherein said homogenous coating mixture is applied to a gold-coated substrate.

44. The method of claim 43, whereby the substrate is gold-coated glass, gold coated ceramic, gold-coated glass-ceramic or a gold-coated polymeric substrate.

45. A method as in claim 36, further comprising examining the coated substrate for uniformity of fluorescence in the 300 to 550 nm wavelength region.

46. The method of claim 36, wherein the fluorescent labeled compound is  
N- (Triethoxysilylpropyl) dansylamide,  
5-Dimethylamino-N-(3-Triethoxysilylpropyl)naphthalene-1-sulfonamide,  
N-Triethoxysilylpropylquinineurethane,  
3-(2,4-Dinitrophenylamino)propyltriethoxysilane, or mixtures thereof.

47 The method of claim 36, wherein the substrate is a substrate for  
DNA, protein function, protein capture, carbohydrate, or tissue microarray  
applications.

48. The method of claim 40, wherein the chemically functional coating is an  
alkylalkoxysilane monolayer.

49. The method of claim 36, wherein the chemically functional coating is an  
alkanethiol monolayer.

50. A method according to claim 36, wherein the homogenous coating mixture  
is applied to the substrate by chemical vapor deposition, sputtering, dip coating,  
ion beam deposition, flame hydrolysis deposition, laser pyrolysis deposition,  
liquid phase deposition, electron beam deposition, plasma arc deposition or flash  
evaporation deposition.

51. A method according to claim 36, wherein the substrate is a low self-fluorescent multi-oxide component glass.

52. A method according to claim 51, wherein the substrate is a low self-fluorescent borosilicate or soda lime silicate glass.

53. A method according to claim 36, wherein the chemically functional coating is a multiaminoalkylmonoalkoxy silane, multiaminoalkyldialkoxysilane, and/or a multiaminoalkyltrialkoxysilane.

54. A method according to claim 53, wherein the multiamino organosilane is trimethoxysilylpropyl-diethylenetriamine(DETA), N-(2-aminoethyl)-3-aminopropyltrimethoxysilane(EDA), (aminoethyl aminomethyl)phenethyltrimethoxysilane (PEDA), or mixtures thereof.

55. A method according to claim 42, wherein said glass comprises, in % by weight on an oxide basis:

SiO <sub>2</sub>	58-85
B <sub>2</sub> O <sub>3</sub>	7 –15
Al <sub>2</sub> O <sub>3</sub>	0 – 8
Na <sub>2</sub> O	0- 15

$K_2O$	0 – 8
$ZnO$	0 – 8
$CaO$	0 – 8
$MgO$	0 – 8
$As_2O_3$	0-2
$Sb_2O_3$	0-2.

56. A method according to claim 42, wherein said glass comprises, in % by weight on an oxide basis:

$SiO_2$	40 - 60
$B_2O_3$	10-20
$Al_2O_3$	8-20
$BaO$	20-30
$Na_2O$	0-5
$K_2O$	0-5
$ZnO$	0-7
$CaO$	0-8
$MgO$	0-5
$As_2O_3$	0-2
$Sb_2O_3$	0-2.

57. A method according to claim 42, wherein said glass comprises, in % by weight on an oxide basis:

SiO <sub>2</sub>	60-70
B <sub>2</sub> O <sub>3</sub>	5-10
Al <sub>2</sub> O <sub>3</sub>	0.1-8
Na <sub>2</sub> O	0-8
K <sub>2</sub> O	0-8
ZnO	3-10
TiO <sub>2</sub>	1-10
CaO	0-5
MgO	0-5
As <sub>2</sub> O <sub>3</sub>	0-2
Sb <sub>2</sub> O <sub>3</sub>	0-2.

58. A method according to claim 42, wherein said glass comprises, in % by weight on an oxide basis:

SiO <sub>2</sub>	65-75
Na <sub>2</sub> O	5-15
K <sub>2</sub> O	5-15
ZnO	2-6
TiO <sub>2</sub>	0.1-5
BaO	0.1-5
CaO	0-10
MgO	0-6
PbO	0-3

$\text{Al}_2\text{O}_3$	0-3
$\text{B}_2\text{O}_3$	0-5
$\text{As}_2\text{O}_3$	0-2
$\text{Sb}_2\text{O}_3$	0-2.

59. An array of immobilized carbohydrate molecules comprising a plurality of carbohydrate molecules attached to a substrate, the uniformity of said molecules on said substrate having been determined by the method of claim 36.

60. An article comprising a self-assembled mixed monolayer on a surface, said mixed monolayer comprising a homogeneous mixture of a fluorescent-labeled compound and a chemically functional compound to which other chemical moieties can be bound when said functional compound is in said self-assembled mixed monolayer.

61. A method of preparing a coated substrate whose coating uniformity can be nondestructively determined comprising:

coating the substrate with a homogenous coating mixture comprising

(a) a labeled compound

and

(b) a chemically functional compound

other molecules being chemically bondable to said functional compound when in said coating.

62. An array of immobilized nucleic acids comprising a plurality of nucleic acid molecules attached to a surface according to claim 1.

63. A surface for attachment of molecules comprising:

(a) a substrate; and

(b) a coating thereon of a homogeneous mixture of

a labeled compound

and

hydrogel polymer,

other molecules being chemically bondable to said hydrogel polymer when in said coating.

64. A surface according to claim 63, wherein the hydrogel polymer contains amine groups.

65. A surface according to claim 64, wherein a portion of the amine groups is reacted with fluorophore compounds.

66. A surface according to claim 64, wherein a portion of the amine containing polymers of the hydrogel contains a fluorophore and is homogenously mixed with non-derivatized amine polymers.



67. A surface according to claim 63, wherein the labeled compound is not chemically bound to the hydrogel.
68. A surface according to claim 63, wherein the labeled compound is fluorescent.
69. A surface according to claim 63, wherein the fluorescent compound is trapped in a homogenous gel matrix formed by said hydrogel polymers.
70. A method of performing a hybridization assay comprising incubating target nucleic acid molecules with a surface according to claim 63, wherein the biomolecules are nucleic acid oligomers.
71. A method of performing a hybridization assay according to claim 70, wherein the fluorescent compound is removed from the surface before conducting the assay.
72. A method of performing a hybridization assay according to claim 71, wherein the fluorescent compound is removed from the surface by leaching.

73. An array according to claim 63, wherein the biomolecules are DNA, unmodified nucleic acids, antibodies, antigens, proteins, sugars, carbohydrates or oligonucleotides.

74. An array of immobilized carbohydrate molecules comprising a plurality of carbohydrate molecules attached to a surface according to claim 63.

75. An array of immobilized protein molecules comprising a plurality of protein molecules attached to a surface according to claim 63.

76. An array of immobilized cells comprising a plurality of cells attached to a surface according to claim 63.

77. An array of immobilized small molecules comprising a plurality of small molecules attached to a surface according to claim 63.

78. An array of nucleic acid molecules comprising a plurality of nucleic acid molecules attached to a surface according to claim 63.

79. A method of preparing a coated substrate for microarray applications comprising:

- i) coating the substrate with a homogenous coating mixture comprising  
a fluorescently labeled compound

and

a chemically functional compound

other molecules being chemically bondable to said functional compound

when in said coating,

ii) generating and detecting the fluorescent signal from said fluorescently labeled compound,

and thereafter,

iii) reducing the fluorescent activity of the fluorescent labeled compound.

80. A method according to claim 79, wherein the fluorescent signal is detected to determine the uniformity of the homogenous coating mixture on a substrate.

81. A method according to claim 79, wherein the fluorescent activity of the fluorescent-labeled compound is reduced by photo bleaching.

82. A method according to claim 79, wherein the fluorescent activity of the fluorescent- labeled compound is reduced by heat treatment.

83. A method according to claim 79, wherein the activity of the fluorescent labeled compound is reduced to a level that does not interfere with optical measurements used for microarraying applications.

84. A method according to claim 79, wherein the substrate is a low self-fluorescent glass.

85. A method according to claim 79, wherein the glass is borosilicate or soda lime silicate glass.

86. A method according to claim 79, wherein the fluorescent labeled compound is derived from the reaction of a fluorescent dye and a second chemically functional compound.

87. A method according to claim 79, wherein the chemically functional compound is an organosilane.

88. The method of claim 79, wherein the substrate is for nucleic acid, protein function, protein capture, carbohydrate, small molecule, cell or tissue microarray applications.